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Effects of UV radiation and salinity on the intertidal macroalgae *Palmaria palmata* and *Ulva lactuca*; effects on photosynthetic performance, growth and pigments

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Abstract

The tolerance of *Palmaria palmata* and *Ulva lactuca* from two positions (upper and lower) of the shoreline to low salinity and Ultraviolet radiation (UVR) was assessed using chlorophyll fluorescence, relative growth rates, chlorophyll a (Chla) and carotenoid concentrations. Species differences and position on shore at collection were major factors in the ability to tolerate increases in UVR. Both species were found to have sensitivity to both UVR and low salinities, although *P.palmata* was more sensitive to treatment than *Ulva.* The ability to increase chlorophyll and carotenoid concentrations when under low salinities and Ultraviolet B (UVB) compared to normal salinity was seen in *P.palmata* on the lower shore. This resulted in no further reduction in relative growth rate (RGR) from other light levels which occurred when treated with normal salinity occurred, indicating antagonistic effects. However, on the upper shore the effects were additive, as with decreased Chla and carotenoids. *P.palmata* showed a lower (RGR)(g per day) under UVB, which was not seen under normal salinity. *Ulva* showed sensitivity, but increased photosynthetic efficiency of PSII and growth rates compared to *P.palmata.* Additive effects were also observed in lower shore *Ulva* under UVB and low salinity, whereby the two together caused greater decreases in growth rate. The interactive effects of light and salinity have been seen to be complex with differences within species, between locations and light treatments all governing the action observed, which have been seen to range from antagonistic to additive.

Keywords; Ultraviolet radiation, Salinity, Macroalgae, Growth, Photosynthetic performance, Photosynthetic pigments

Introduction

The stratospheric ozone layer absorbs UVB radiation and so provides protection to all organisms against UVB. Due to the hole in the ozone layer, increases of UVB reach the earth's surface, especially in Antarctic and Arctic areas where the thinning is most prominent. Moreover, irradiance and UV levels fluctuate over seasonal, tidal and daily cycles and thus can produce damage over short and long term periods (Madronich, *et al.* 1998, Michler, *et al.* 2002). Although the hole in the ozone has been shown recently to be repairing gradually, changing climates and extreme weather patterns are now on the forefront of concerns, since not only UVR (Holzinger, *et al.* 2004) but high light levels have also been seen to be damaging to macroalgae (Talarico, *et al.* 2000, Roldela, *et al.* 2005). When considering effects of UVR on macroalgae it is noteworthy to consider; springtime ozone depletion (Karentz, *et al* 1991), adaption to low irradiances over winter months and reproductive/life stages of the algae in question (Cordi, *et al.* 2001).

Many studies have reported detrimental impacts of elevated UV on marine macroalgae which include, but are not limited to; alterations in photosynthetic performance, pigments, growth rates, antioxidant concentrations and ultra-structural changes (Poppe, *et al.* 2002, 2003). The importance of these changes in primary producers as structural habitat providers and as an energy source are highlighted, as shifts in community structure due to tolerant species becoming dominant can cause alterations within the whole ecosystem (Bischof, *et al.* 1998, Hader *et al.* 2007).

High UV and irradiance do not occur in isolation. More often, other stresses such as desiccation and temperature are present. This therefore has to be taken into account when studying such processes to ultimately determine if such influences work in synergy, are additive or antagonistic. Salinity affects algae as osmotic regulation is imperative to maintaining the homeostasis within cells and therefore a healthy state (Eggert, *et al.* 2007). Hyper/hypo-saline conditions can arise due to evaporation, rain fall, river run off into isolated rock pools and throughout estuarine areas. Intertidal algae which inhabit the upper areas of the shore (closest to the high water mark) are more exposed to low salinities, high irradiance and desiccation, due to having a longer time from when the tide retreats until its return at high tide. Salinity causes osmotic pressures and like UVR, causes oxidative stress and production of reactive oxygen species (ROS) which can cause damage to internal structures such as membranes, DNA and proteins (Kumar, *et al.* 2010, Li *et al.* 2010, Ledford, and Niyogi, 2005). It is therefore, no revelation that under salinity stress, algae have been seen to increase antioxidant enzymes and phycobiliproteins concentrations and reduced growth occurs (Kumar, *et al.* 2010). On a genomic scale it has been shown that energy related genes are down-regulated under high light and low salinities (whilst stress genes are up-regulated), further enforcing the theory that these conditions are detrimental to the overall health and growth of the organism (Collen, *et al.* 2007).

The role of chlorophyll and carotenoids are important in the protection against harmful effects experienced due to irradiance (Noguchi, *et al.* 2002). Carotenoids are accessory pigments and have an antioxidant role protecting chlorophyll from degradation via quenching triplet chlorophyll, while secondary carotenoids which act as screening pigments (Franklin and Forester 1997). Under high light these are degraded and thus they need to be replaced in order to maintain protection, this therefore results in an energy trade-off away from growth. This coupled with reduced

photosynthetic efficiency can therefore lead to overall decreases in fitness, growth and productivity.

Two species of algae; *Palmaria palmata* and *Ulva lactuca* were chosen because of their presence on the intertidal shore area. Studies have found the two species to be sensitive to salinity, UV and high irradiances (Liu, *et al.* 2010, Holzinger, *et al.* 2004, Han, *et al.* 2003a, 2003b). For that reason a comparison between two species which inhabit the same area is revealing in the prediction of community structure of the species under supposed conditions.

It was hypothesised that species inhabiting the upper shore would have an increased tolerance to UV irradiances and low salinities than the lower shore. This is reasoned by the upper shore algae being more exposed to high light at low tide and river run off and so defensive/protective mechanisms would already be developed and thus possess a greater tolerance to stress. Decreased tolerance would be represented by lower pigment concentrations, a reduction in photosynthetic performance and consequently growth rate. The aims of this study are to see if changes in salinity cause an increased sensitivity to various light irradiances and how the changes in pigment concentrations, photosynthesis and growth would change and interact.

Materials and Methods

Algal collection

Ulva latuca and *Palmira palmata* were collected from Wembury beach, Devon (50°18' N latitude, -4°05'W longitude), a non-polluted site. Collection at 10m (upper shore) and 30m (lower shore) from the high tide mark at low tide took place on the 24th November 2010 and samples were transferred to the laboratory in darkness. Algae were kept in filtered seawater with constant aeration at 16^{o}c, under a 12:12 light dark regime. Algae was cut into approximately 3cm lengths/diameter and washed with filtered seawater to remove debris/organisms.

Experimental procedure

Samples were placed into separate petri dishes to prevent self-shading and covered (0.5cms) with filtered seawater (normal) or a 50:50 mix of filtered seawater with distilled water (Hypo-saline/low). Petri dishes remained uncovered, static and exposed for 2.5hrs to elevated UVR in a constant temperature cabinet at 16^{o}c. UV irradiation was supplied by UV tubes (Phillips MCFE 40W/33) with a constant background irradiance of PAR supplied from tungsten bulbs. Filters were placed over corresponding dishes to allow different wavelengths of light though; PAR (Ultraphan, removal of UVA and UVB), PAR and UVA (PARA)(Mylar 125 D, removal of UVB) and PAR, UVA and UVB (PARAB)(35mm cellulose diacetate foil). Irradiation was monitored before each exposure using spectroradiometer (MACAM photometrics ltd. Spectroradiometer, model SR991).

Co-ordinates of the site were obtained (Google earth) and were used to find the Dobson units and Spectrum between 280 and 415nm over Wembury on the 24th November 2010 (Total ozone mapping spectrometer, 2009, Quick TUV calculator, 2011). Wavelengths were weighted using the weighting function from Flint and Caldwel (2003) and percent of Ozone reduction relative to the amount of UVB was estimated from Cordi*, et al* (2001) using the computer program of Björn and Murphy (1985).

Photosynthetic efficiency

On arrival at the laboratory and during experimental procedures chlorophyll fluorescence was measured using a non-modulated Plant Efficiency Analyser™ (Hansatech Instruments Ltd.). Dark adaption time was established and all samples were subsequently dark adapted for 20mins. Saturating irradiance was produced by six high-irradiance light emitting diodes (LEDs)(Hansatech Instruments Ltd). Fluorescence was initiated by a 1s red light pulse with an irradiance at 3000 µmol m^{-2} s^{-1}. Autogain was calibrated before each set of measurements to produce maximum fluorescence values.

Optimal quantum yield (F_v / F_m) was calculated whereby; $F_v = F_m - F_0$

F_v = Variable fluorescence, F_m = Maximal fluorescence and F_0 = initial fluorescence.

Growth rates

Thallus area

Samples were measured using a leaf area analyser (Delta-T Image Analysis System type DIAS) at 0, 3, 24, 27, 48 and 72hrs. Relative growth rate was determined by the following equation;

$$\text{RGR (cm}^2\text{ per day)} = \log(At_x) - \log(At_0)/\ t*100$$

(A= Area in cm^2, At_x= cm_2 at x days, At_0= cm_2at 0 days, t= days)

Weight

Samples were blotted dry and weighed at 0, 3, 24, 27, 48 and 72hrs. Relative growth rate was determined by the following equation;

$$\text{RGR (g per day)} = \log(Wt_x) - \log(Wt_0)/\ t*100$$

(W= Weight in grams, Wt_x= g at x days, Wt_0= g at 0 days, t= days)

Pigment analysis

Samples were frozen after second treatment (at 27hrs) and kept at -80^{o}c until analysis. Chlorophyll was extracted from 0.06g of tissue using 1ml of dimethylsulfoxide (DMSO), at 40^{o}c for 30min. A pestle was used to grind samples at 0min and 15min where a further 0.25ml of DMSO was added after 15minutes. Samples were centrifuged at 10,000 RPM for 10min. For each treatment triplicate samples were analysed for pigments and quantified spectrophotometrically (Helios Epsilon visible spectrophotometer) according to Wellburn (1994).

Chlorophyll a (C_a) =$12.19*(A_{665})-3.45*(A_{648})$

Chlorophyll b (C_b) = $21.99*(A_{648})-5.32*(A_{665})$

Total Carotenoid = $((1000*A_{480})-2.14*(C_a)-70.16*(C_b))/220$

Statistical analysis

Mean values, standard deviations and standard errors were calculated, statistical significance was tested with a one-way ANOVA's, whereby $P<0.05$ was considered to be statistically significant. Tukey tests were carried out on significant tests and confidence levels generated.

Results

Samples were exposed to three light levels PAR, PARA and PARAB (Fig.1). PARAB spectrum is clearly greater than the other two with slightly higher peaks at 430nm, 560nm and 580nm. PARA contains PAR and UVA radiation but has a lower intensity UVA than the wavelength containing UVB. PAR contains no wavelength in the ultraviolet range. Wavelength taken from Wembury shows no UVB radiation but increases quite dramatically within the UVA range. It was found that at the time of sampling the level of ozone at the site was 331 Dobson Units.

Intensity of light used in the experiment and from the collection site is shown in Table1. PARAB, as expected has an increased weighted UVB value than the other three. The light intensity is increased slightly between light treatments. Corresponding Ozone depletion percentages are estimates taken from Cordi, et al. (2001) which used the computer program of Björn and Murphy, (1985).

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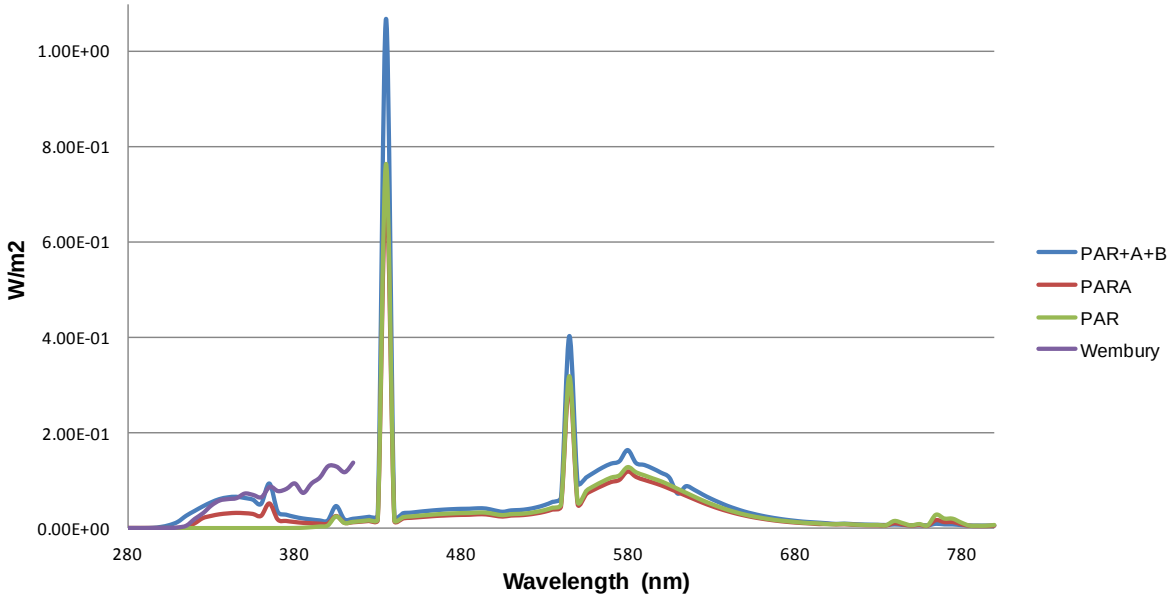

Figure 1: Spectrum showing irradiance levels for the three light treatments PAR, PARA and PARAB. Also show irradiance levels up to 415nm wavelength at the collection site, Wembury, on the 24th November 2010 at 13.30hrs

Table1: Exposure to irradiances within the experiment

	Unweighted irradiance (Wm^{-2})	Weighted UVB irradiance (Wm^{-2})	% Ozone depletion
Wembury	1.87	0.129	0%
PAR	1.92	0.113	0%
PARA	1.97	0.135	0%
PARAB	2.29	0.643	31-35%

Unweighted irradiance and weighted UVB irradiance, calculated using spectra up to 315nm according to Flint and Caldwel (2003). Ozone depletion was estimated using Cordi, *et al.* (2001).

Photosynthetic efficiency

Fv/Fm values were compared between controls with the values taken after the first treatment (Table2). *Ulva lactuca* sampled from the upper shore did not show any change in Fv/fm values in either salinity concentrations. From the lower shore, *Ulva lactuca* had significant differences when exposed to PARA under both salinity treatments (P=0.028, $F_{1,76}$=5.01) (P=0.043, $F_{1,76}$=4.25) and PARAB only when under low salinity treatments (P=0.043, $F_{1,76}$=4.24).

Table 2: *F*v/*F*m values post treatment, compared with control

Ulva lactuca

	Upper						Lower					
	Normal			Low			Normal			Low		
	PAR	**PARA**	**PARAB**	**PAR**	**PARA**	**PARAB**	**PAR**	**PARA**	**PARAB**	**PAR**	**PARA**	**PARAB**
Control (*F* v/*F* v)	0.688	0.688	0.688	0.688	0.688	0.688	0.752	0.752	0.752	0.752	0.752	0.752
	± 0.019	± 0.019	± 0.019	± 0.019	± 0.019	± 0.019	±0.018	±0.018	±0.018	±0.018	±0.018	±0.018
After 2.5hrs treatment (Fv/Fv)	0.670	0.668	0.608	0.612	0.708	0.616	0.812	0.640	0.645	0.813	0.760	0.650
	± 0.068	± 0.078	± 0.055	± 0.074	± 0.060	± 0.047	± 0.028	± 0.054	± 0.064	± 0.028	± 0.064	± 0.053
P value	NS	NS	NS	NS	NS	NS	NS	0.028	NS	NS	0.043	0.043
$F_{1,76}$	NS	NS	NS	NS	NS	NS	NS	5.01	NS	NS	4.25	4.24

P.palmata

	Upper						Lower					
	Normal			Low			Normal			Low		
	PAR	**PARA**	**PARAB**	**PAR**	**PARA**	**PARAB**	**PAR**	**PARA**	**PARAB**	**PAR**	**PARA**	**PARAB**
Control (Fv/Fv)	0.760	0.760	0.760	0.760	0.760	0.760	0.794	0.794	0.794	0.794	0.794	0.794
	±0.008	±0.008	±0.008	±0.008	±0.008	±0.008	±0.004	±0.004	±0.004	±0.004	±0.004	±0.004
After 2.5hrs treatment (Fv/Fv)	0.714	0.679	0.605	0.741	0.678	0.624	0.736	0.678	0.644	0.722	0.690	0.661
	±0.010	±0.078	±0.033	±0.016	±0.028	±0.021	±0.016	±0.034	±0.021	±0.011	±0.022	±0.019
P value	0.020	0.001	0.000	NS	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
$F_{1,76}$	5.65	13.18	45.05	NS	13.97	43.35	19.36	43.38	110.93	34.44	50.64	93.37

Mean Fv/fm values of controls (no treatment) and after the first 2.5hrs of treatment with different irradiances and salinities (±SE) in *Ulva lactuca* and *P.palmata*. P values and F values are shown for significant treatments, generated by one way ANOVERS. P< 0.050 was considered significant and SN for non-specific values. Tukey tests were performed and a confidence level of 95% was established.

P.palmata showed greater overall changes between the controls and post-treatment values, with only samples collected from the upper shore under PAR and low salinity showing no significant difference. All lower shore samples were significantly different (P=0.000, $F_{1,76}$=19.36-110.93). Upper samples under normal salinity saw a decreased P-value from PAR (P=0.020, $F_{1,76}$=5.65) to PARA (P=0.001, $F_{1,76}$=13.18) and PARAB(P=0.000, $F_{1,76}$=45.05), PARAB having the lowest. This suggests a decrease in Fv/Fm as the irradiance and UV levels increases. Samples treated with low salinity were significant with PARA (P=0.000, $F_{1,76}$=25.35) and PARAB (P=0.000, $F_{1,76}$=25.35) irradiances.

In comparison of *F*v/*F*m between positions, controls for both *Ulva* (P=0.016, $F_{1,131}$= 5.91) and *P.palmata* (P=0.000 $F_{1,131}$= 14.64) were different (Fig.2). After treatment with PAR *Ulva* showed lower *F*v/*F*m values for upper shore positions, whereas lower shore samples saw an increase in *F*v/*F*m after treatments (Fig.3). However, difference in positions was only found significant in samples from the upper shore under low salinity treatment (PAR P=0.020 $F_{1,21}$= 6.38) with no difference between the control and after treatment values seen. There was no significant difference between the normal and low salinity treatments in *F*v/*F*m after the first exposure of 2.5hrs in any of the tests. Between species both upper (P=0.001 $F_{1,131}$= 12.48) and lower (P=0.028 $F_{1,131}$= 4.94) shore samples showed significant differences between species in control groups.

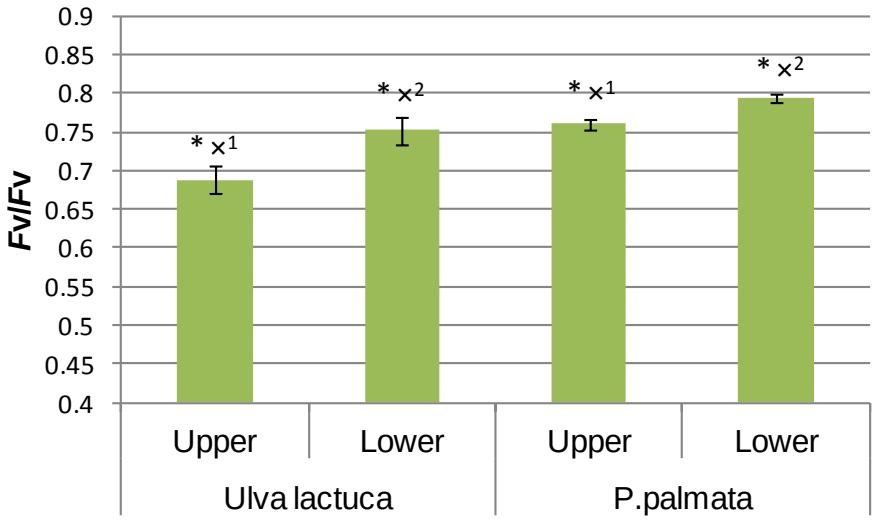

Figure 2: Comparison of Fv/Fv values in controls

Mean *F*v/*F*m values (±SE) from 131 control samples for each group are shown. * indicates significant treatments between positions of each species, × indicates significance between species from each location (×[1]=upper, ×[2]= lower). Significance was generated by one way ANOVERS. P< 0.050 was considered significant. Tukey tests were performed and a confidence level of 95% was established.

After treatment, only lower shore samples treated with PAR still showed significance between the two species, both under normal (P=0.030 $F_{1,21}$= 5.44 95%) and low (P=0.007 $F_{1,21}$= 8.95 95%) salinity treatments (Fig 3). This is due to the high values that *Ulva* experienced under these treatments, and suggests an evening-out between species.

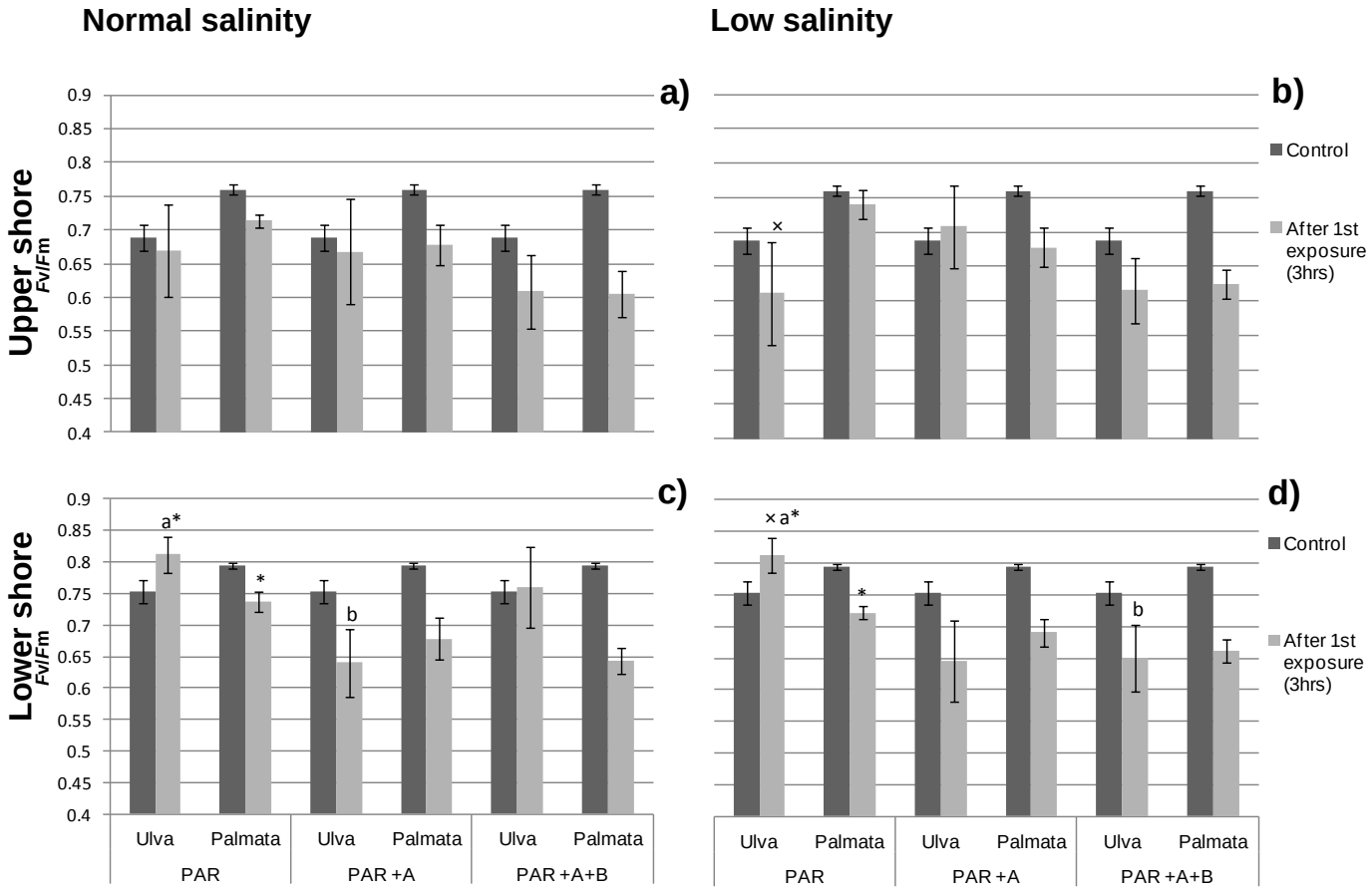

Figure 3: Species comparison of Fv/fm

Species comparison between mean Fv/Fm values (±SE) after exposure to 2.5 hrs of treatment under normal (a,c) and low(b,d) salinity of sample taken from upper (a,b) and lower (c,d) positions of the shore sunder different light treatments (PAR, PARA, PARAB). Significance between positions is indicated, whereby * P<0.050, ** P<0.005, ***P=0.000.

Changes of Fv/fm value due to light treatments in *Ulva* only showed changes in lower shore samples, namely PARA showing a decrease from PAR (P=0.010 F=1,21 7.98) after normal salinity treatment PARA (P=0.026 $F_{1,21}$= 5.76) and PARAB (P=0.013 $F_{1,21}$= 7.48) experiencing lower values to PAR with low salinity. *P.palmata* exhibited greater changes due to light irradiance with all samples from both positions and under both salinities having significant differences. Overall differences in irradiance was seen in both upper and lower normal salinity treatments. Specifically, all samples showed a decreased Fv/Fm value after PARAB irradiance compared to PAR, in upper shore normal (P=0.005 $F_{1,21}$= 9.80) and low salinities (P=0.000

$F_{1,21}$=19.52), additionally lower shore normal (P=0.002 $F_{1,21}$=12.24) and low salinities (P=0.011 $F_{1,21}$=7.81).

Growth rates

Thallus area

There were few significant changes found between the RGR calculated using area (cm^2). Within species, differences between positions and salinity treatments were only detected in *P.palmata,* in which the lower shore samples had a significantly lower RGR (P=0.003, $F_{1,21}$=11.43) than the upper shore when treated with normal salinity and PARA irradiance. *Ulva* showed more growth than *P.palmata.*, Significantly increased growth rates (cm^2 per day) were observed in *Ulva* from samples collected from the lower shore and exposed to PAR (normal salinity P=0.000, $F_{1,12}$=17.30, low salinity P=0.032, $F_{1,12}$=5.34) and PARA(normal salinity P=0.002, $F_{1,12}$=12.31 and low salinity P=0.001, $F_{1,12}$=16.60). There was also significance between species collected from the upper shore under normal salinity and PAR treatments (P=0.001, $F_{1,12}$=15.00) (Fig 4).

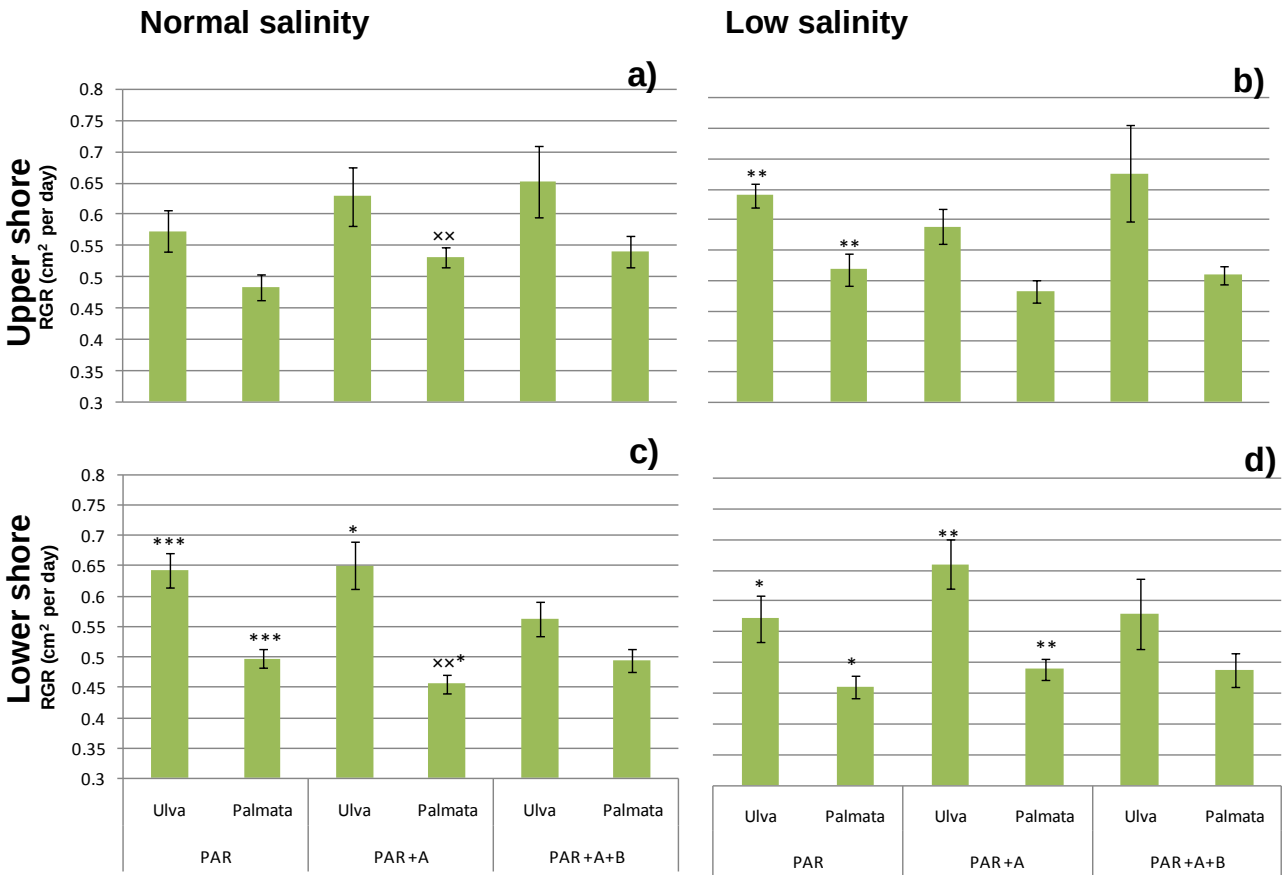

Figure 4:. Comparison of RGR (cm^2 per day)

Mean RGR (cm^2 per day) (±SE) of *Ulva lactuca* and *P.palmata* after exposure to 2.5 hrs of treatment under normal (a,c) and half (b,d) salinity of sample taken from upper (a,b) and lower (c,d) positions of the shore under different light treatments (PAR, PARA, PARAB).× indicated significance in position of sample treatment and * indicates significance between positions. Value of significance is indicated by number of */× whereby; 1 = P<0.050, 2 = P<0.005 and 3 =P=0.000. . Tukey tests were performed and a confidence level of 95% was established.

Weight

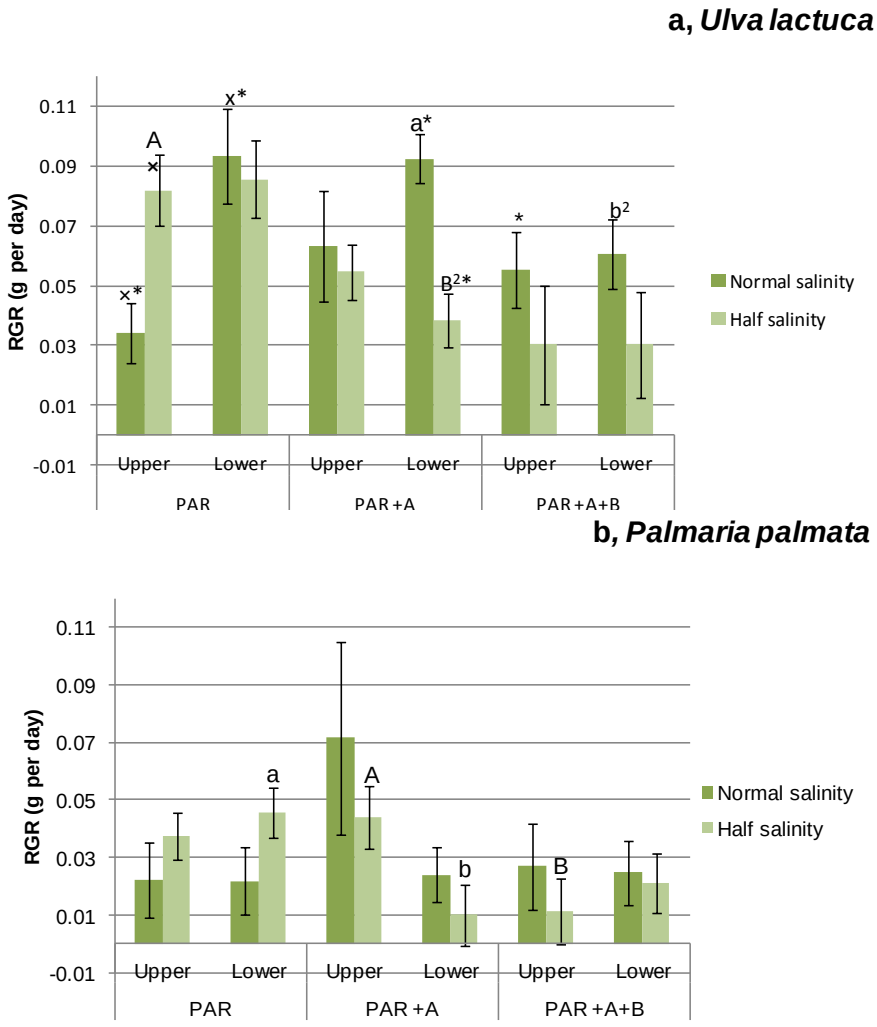

Figure 5: RGR of *Ulva* and *P.palmata,* comparison of position and salinity,

Mean RGR (g per day) (±SE) of i) *Ulva* and ii) *P.palmata* after exposure to 2.5 hrs of treatment under normal and low salinity from samples taken from upper and lower positions of the shore under different light treatments (PAR, PARA, PARAB). × indicates significance in position of sample treatments, * indicates significance between salinities and letters (aA/bB) indicate significance between light treatments. Value of significance is indicated by number of */× whereby; 1 = P<0.050, 2 = P<0.005 and 3 =P=0.000. . Tukey tests were performed and a confidence level of 95% was established.

Overall the RGR calculated from weight exhibited greater variability than the RGR calculated using area. RGR was significantly reduced (P=0.025, $F_{1,12}$=5.88) in *Ulva* collected from the upper shore, treated with half salinity and PARAB irradiance compared to those treated with PAR. There was no significance however between PAR with either PARA or PARAB. *Ulva* collected from the lower shore showed a significant increase in RGR under PAR conditions compared to PARA in under half salinity (P=0.004, $F_{1,12}$=10.76). Whereas, under normal salinity there was a significant increase in PARA to PARAB (P=0.036, $F_{1,12}$=5.06)(Fig.5i).

P.palmata showed significant decrease when exposed to higher UV, but, only when treated with low salinity also. Significant decreases in PAR and PARA from lower shore were found (P=0.032, $F_{1,12}$=4.70) as well as in upper shore samples between PARA and PARAB (P=0.032, $F_{1,12}$=5.34)(Fig.5ii).

Upper shore samples of *Ulva* showed a lower growth rate in all irradiance treatment under normal salinity compare to lower shore samples. However, the growth rate was only significantly lower in PAR irradiance treatments (P=0.006, $F_{1,12}$=9.54)(Fig.5i). There were no differences within *P.palmata* positions.

Under low salinity *P.palmata* exhibited a lower RGR than *Ulva* where under PAR irradiance this was significant in samples collected from both lower (P=0.008, $F_{1,12}$=8.83) and upper positions (P=0.008, $F_{1,12}$=8.66) of the shore (Fig.6). Under normal salinity the lower samples of *P.palmata* were significantly lower than *Ulva* when exposed to all irradiances; PAR (P=0.002, $F_{1,12}$=11.99), PARA (P=0.000, $F_{1,12}$=26.97) and PARAB(P=0.047, $F_{1,12}$=4.46), whereas samples from the upper shore showed no significant differences (Fig.6).

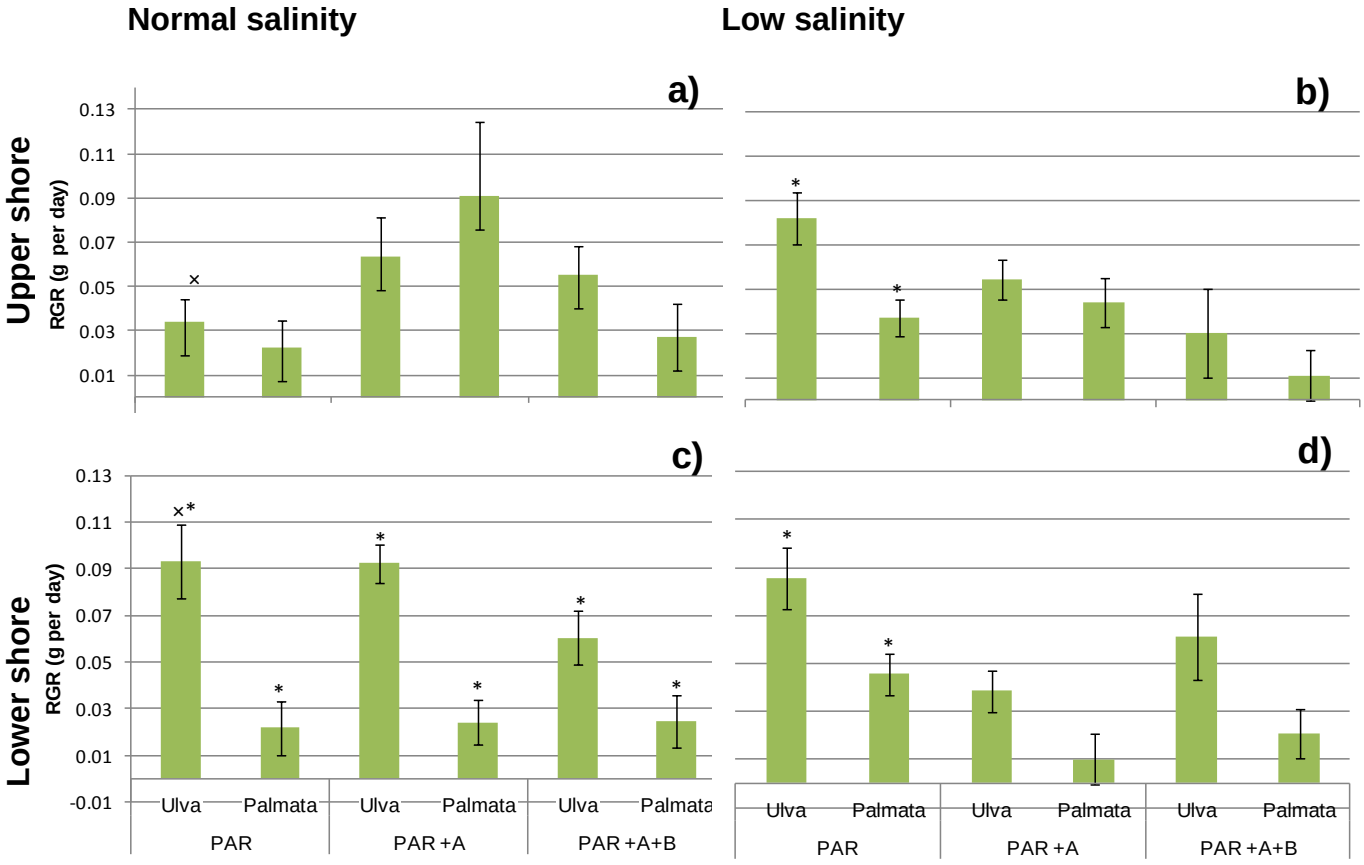

Figure 6: Mean RGR (g per day)(±SE) under normal (a,c) and half salinity (b,d) of sample taken from upper (a,b) and lower (c,d) positions of the shore under different light treatments (PAR, PARA, PARAB). × indicated significance in position of sample treatment and * indicates significance between species. Value of significance is indicated by number of */× whereby; 1 = P<0.050, 2 = P<0.005 and 3 =P=0.000

Pigment analysis

Table 3, *Ulva* collected from the upper shore only showed a significant increase in Chla when under low salinity and PARAB light irradiances (P<0.050). However, samples from the lower shore with normal salinity exhibited significantly different levels of Chla under all light irradiances (P=0.001) from the lowest in PAR to PARAB, PARA with the highest levels being found in the control samples (P<0.005). Under low salinity all light levels were significantly lower than the control also (P<0.050), where PARA and PARAB are further significant from PAR (P=0.019, P=0.011).
Upper shore *P.palmata* showed significant reductions in Chla concentration under all light treatments compared to the upper shore control (P<0.010). Only between PAR and PARAB of the low salinity treatment was found to be different with the PAR treatment having a reduced Chla compared to PARAB (P=0.044). The controls of the lower shore samples were not significantly different to any of the treatments. Between light treatments of the samples under normal salinity PARAB was found to have significantly increased Chla concentration than that of PAR (P=0.00 $F=_{1,5}$ 137.71) and PARA (P=0.001, $F=_{1,5}$ 75.32) (Table3).

Table 3: Chlorophyll a and total Carotenoid concentration, significance of light treatment between controls and post treatment values

Chlorophyll a (µg/m^{-1})							
Species	**Position**	**Salinity**	**Control**	**PAR**	**PARA**	**PARAB**	**Pvalue**
Ulva lactuca	**Upper**	Normal	13.3 ±6.7	15.9 ±0.2	16.2 ±0.2	15.9 ±0.4	NS
		Low	13.3 ±6.7	14.0 ±0.4^{a}	12.3 ±1.3^{a}	18.3 ±1.3^{b}	<0.050
	Lower	Normal	21.1 ±4.3^{a}	3.1 ±0.5^{b}	7.4 ±0.3^{b}	5.7 ±0.4^{c}	<0.050
		Low	21.1 ±4.3^{a}	10.7 ±1.0^{b}	5.9 ±0.8^{c}	6.1 ±0.2^{d}	<0.050
P.palmata	**Upper**	Normal	8.6±1.1^{a}	4.3±0.5^{b}	4.0±0.2^{b}	4.2±0.4^{b}	<0.001
		Low	8.6±1.1^{a}	3.0±0.5^{b}	3.9±0.2^{b}	4.9±0.4^{c}	<0.050
	Lower	Normal	13.3±2.7	3.7±0.0^{a}	3.5±0.1^{a}	4.1±0.0^{b}	<0.001
		Low	13.3±2.7	6.4±1.2	6.8±0.3	7.2±0.2	NS
Total Carotenoids (µg/m^{-1})							
Ulva lactuca	**Upper**	Normal	1.5±0.2^{a}	2.0±0.2	1.8±0.4	2.4±0.2^{b}	<0.050
		Low	1.5±0.2^{a}	2.3±0.4	1.8±0.3^{a}	2.7±0.1^{b}	<0.050
	Lower	Normal	1.8±0.1	1.6±0.0	1.6±0.1	1.3±0.3	NS
		Low	1.8±0.1^{a}	4.6±0.1^{b}	1.4±0.2^{a}	1.3±0.2^{a}	0.000
P.palmata	**Upper**	Normal	3.2±0.2^{a}	1.9±0.2^{b}	1.8±0.1^{b}	1.9±0.2^{b}	<0.005
		Low	3.2±0.2^{a}	1.4±0.2^{b}	1.6±0.2^{b}	1.9±0.2^{b}	<0.010
	Lower	Normal	1.8±0.1	2.6±0.2^{a}	1.9±0.3	1.9±0.1^{b}	<0.050
		Low	1.8±0.1^{a}	2.3±0.2	1.8±0.2	3.2±0.2^{b}	<0.050

Control and post exposure values for Chla and total Carotenoid concentration (µg/m^{-1}). Mean values have been taken (±SE) and P values calculated (one way ANOVA). Letters (^{a,b,c,d}) represent significantly different groups of irradiance within each treatment.

Table 4. Comparison of positions on shore in Chlorophyll a and total Carotenoid concentration

		Upper			Lower		
Chlorophyll a (µg/m-1)		**PAR**	**PARA**	**PARAB**	**PAR**	**PARA**	**PARAB**
Ulva lactuca	**Normal**	15.9 ±0.2*	16.2 ±0.2*	15.9 ±0.4*	3.1 ±0.5**	7.4 ±0.3	5.7 ±0.4
	Low	14.0 ±0.4*	12.3 ±1.3*	18.3 ±1.3	10.7 ±1.0**	5.9 ±0.8	6.1 ±0.2
	P-value	0.011	0.040	NS	0.003	NS	NS
	F1,5	19.82	22.28	NS	44.49	NS	NS
P.palmata	**Normal**	4.3±0.5	4.0±0.2	4.2±0.4	3.7±0.0	3.5±0.1**	4.1±0.0***
	Low	3.0±0.5	3.9±0.2	4.9±0.4	6.4±1.2	6.8±0.3**	7.2±0.2***
	P-value	NS	NS	NS	NS	0.001	0.000
	F1,5	NS	NS	NS	NS	93.82	176.35
Total Carotenoids (µg/m-1)							
Ulva lactuca	**Normal**	2.0±0.2	1.8±0.4	2.4±0.2	1.6±0.0**	1.6±0.1	1.3±0.3
	Low	2.3±0.4	1.8±0.3	2.7±0.1	4.6±0.1**	1.4±0.2	1.3±0.2
	P-value	NS	NS	NS	0.002	NS	NS
	F1,5	NS	NS	NS	47.71	NS	NS
P.palmata	**Normal**	1.9±0.2	1.8±0.1	1.9±0.2	2.6±0.2	1.9±0.3	1.9±0.1*
	Low	1.4±0.2	1.6±0.2	1.9±0.2	2.3±0.2	1.8±0.2	3.2±0.2*
	P-value	NS	NS	NS	NS	NS	0.017
	F1,5	NS	NS	NS	NS	NS	25.35

Significance between positions to the concentration of Chlorophyll a and total Carotenoids in *Ulva lactuca* and *P.palmata* in controls and after two exposures of 2.5hrs to treatments (PAR, PAR+UVA, PAR+UVA+UVB). Means are shown (±SE) with P and F values calculated using one way ANOVERS. Significance is indicated whereby * P<0.050, ** P<0.005, ***P=0.000

Comparisons between upper and lower shore are represented in Table 4. Neither species were found to have significantly different Chla between positions, with only *P.palmata* showing increased total carotenoid concentration in the upper shore (P=0.006, F1,5 29.38). All upper shore samples of *Ulva* demonstrated significantly higher Chla concentrations. However, only after treating *P.palmata* with UV irradiance in low salinity conditions was an increase seen in lower shore samples compared to upper in the Chla concentrations (PARA P=0.005, PARAB P=0.006).

Low salinity increases the chlorophyll concentration in upper shore *Ulva* under PAR (P=0.011, $F_{1,5}$=19.82) and PARA (P=0.040, $F_{1,5}$=22.28) irradiances compared to normal salinity. Whereas lower shore samples under PAR (P=0.003, $F_{1,5}$=44.49) saw increases under normal salinity. *P.palmata* collected from the lower shore also showed increases with normal salinity but in PARA (P=0.001, $F_{1,5}$=93.82) and PARAB(P=0.000, $F_{1,5}$= 176.35). Total Carotenoid concentration only differed in lower shore samples of *Ulva* under PAR (P=0.002, $F_{1,5}$=47.71) and *P.palmata* under PARAB (P=0.017, $F_{1,5}$=25.35).

A significant difference in Chla concentration between species collected from the lower shore (P=0.005) was seen (Table 6). All upper shore samples (except the control) showed a significance (P<0.003) between species. Samples collected at the lower shore exhibited differences between species in the control (P=0.005), after exposure to PARA under normal salinity (P=0.000) and exposure to PARAB under normal (P=0.016) and low (P=0.021) salinity conditions. Differences in total carotenoid concentrations were seen in controls from the upper shore (P=0.005) and after upper shore samples were treated with PARAB and low salinity (P=0,021). Lower shore samples showed a significant change among the two species after exposure to PAR under normal salinity (P=0.000) and PARAB under both normal (P=0.041) and low (P=0.003) salinities.

Table 5: Significance between salinity treatments to the Chlorophyll a and total Carotenoid concentration of *Ulva lactuca* and *P.palmata*

Chlorophyll a (µg/m-1)			**Normal**			**Low**		
		Control	**PAR**	**PARA**	**PARAB**	**PAR**	**PARA**	**PARAB**
Ulva lactuca	**Upper**	13.3 ±6.7	15.9 ±0.2***	16.2 ±0.2***	15.9 ±0.4***	14.0 ±0.4	12.3 ±1.3*	18.3 ±1.3**
	Lower	21.1 ±4.3	3.1 ±0.5***	7.4 ±0.3***	5.7 ±0.4***	10.7 ±1.0	5.9 ±0.8*	6.1 ±0.2**
	P-value	NS	0.000	0.000	0.000	0.033	0.012	0.001
	$F_{1,5}$	NS	492.1	516.51	327.77	10.12	18.88	89.55
P.palmata	**Upper**	8.6±1.1	4.3±0.5	4.0±0.2	4.2±0.4	3.0±0.5	3.9±0.2**	4.9±0.4*
	Lower	13.3±2.7	3.7±0.0	3.5±0.1	4.1±0.0	6.4±1.2	6.8±0.3**	7.2±0.2*
	P-value	NS	NS	NS	NS	NS	0.005	0.006
	$F_{1,5}$	NS	NS	NS	NS	NS	31.52	28.78
Total Carotenoids (µg/m^{-1})								
Ulva lactuca	**Upper**	1.5±0.2	2.0±0.2	1.8±0.4	2.4±0.2*	2.3±0.4**	1.8±0.3	2.7±0.1**
	Lower	1.8±0.1	1.6±0.0	1.6±0.1	1.3±0.3*	4.6±0.1**	1.4±0.2	1.3±0.2**
	P-value	NS	NS	NS	0.014	0.005	NS	0.003
	$F_{1,5}$	NS	NS	NS	17.34	31.2	NS	43.71
P.palmata	**Upper**	3.2±0.2*	1.9±0.2*	1.8±0.1	1.9±0.2	1.4±0.2*	1.6±0.2	1.9±0.2
	Lower	1.8±0.1*	2.6±0.2*	1.9±0.3	1.9±0.1	2.3±0.2*	1.8±0.2	3.2±0.2
	P-value	0.006	0.045	NS	NS	0.017	NS	0.017
	$F_{1,5}$	29.38	8.3	NS	NS	15.62	NS	15.58

Comparisons of controls and samples treated under PAR, PARA and PARAB irradiances, normal/half salinity for two exposures of 2.5hrs over 27hrs total time. ^{U}/^{P} indicate the species which has the higher concentration (^{U} =*Ulva*, ^{P} =*P.palmata*). P-values are shown for significant values (one-way ANOVER), P< 0.050 were considered significant.

Table 6: Chlorophyll a and Total Carotenoid differences between species collected from upper and lower positions of the shore.

Chlorophyll a (µg/m-1)		**Normal**			**Half**		
	Control	**PAR**	**PARA**	**PARAB**	**PAR**	**PARA**	**PARAB**
Upper	NS	0.000^{U}	0.000^{U}	0.000^{U}	0.000^{U}	0.003^{U}	0.001^{U}
Lower	0.005^{U}	NS	0.000^{U}	0.016^{U}	NS	NS	0.021^{P}
Total Carotenoids (µg/m^{-1})							
Upper	0.002^{P}	NS	NS	NS	NS	NS	0.033^{U}
Lower	NS	NS	NS	0.041^{P}	0.000^{U}	NS	0.003^{P}

Means for each treatment are shown (±SE), with P and F values calculated using one way ANOVERS. Significance is indicated by whereby * P<0.050, ** P<0.005, ***P=0.000.

Discussion

This study set out to find if the effects of UVR and salinity were detrimental and if the combination of both are additive, synergistic or antagonistic. It was found that both stressors have adverse effects to varying extents but the interactions between them are complex as many factors, intrinsic and extrinsic to the algae are involved in determining the sensitivity of the algae and interactions between the stressors.

As in other studies (Johansson, *et al .*2002) this study supports the opinion that algae from different positions of the intertidal shore have different tolerances to UVR, shown here in terms of photosynthetic efficiency of PSII and RGR (g per day). It was hypothesised that this was due to acclimation and an increased tolerance of the algae to high light due to previous exposure to these conditions and/or an inherent tolerance. This facilitates the ability to grow at higher shore levels, so species with effective protective mechanisms are dominant on the upper shore, increasing in sensitivity with depth (Hoyer, *et al.* 2001, Johansson, *et al.* 2002). Adaptations such as the increased protection via carotenoids were shown to increase in *Ulva* under exposure to UVB in both shore positions, whereas *P.palmata* which was seen to be more sensitive in photosynthetic ability only increased concentration to UVB in the lower shore, half salinity treatment.

Ulva exhibited an increase in chlorophyll within all upper shore samples compared to lower, post treatment. This shows that *Ulva* has developed ways in which it can protect against degradation of chlorophyll under high irradiance and therefore demonstrates a level of tolerance*. P.palmata* showed decreases in chlorophyll only after PARA in both salinities in samples from the upper shore, compared to lower shore samples, indicating that upper shore algae saw an increase in the bleaching of pigments compared to lower shore samples. However, increased carotenoid concentration in the lower shore samples could be attributed to induction by UVA and therefore causing increases in the carotenoid:chlorophyll ratio (Jahnke, *et al.* 1999). *Ulva* is better adapted to life on the upper shore due to increase growth compared to *Palmata*, less reduction of photosynthetic ability, increases in carotenoids after UVB exposure and a greater Chla concentration after all treatments.

Both species experienced decreases in photosynthetic ability as irradiance increased. *P.palmata* saw decreases in all but PAR (low salinity), whereas *Ulva* only experienced decreases under high light intensities. This furthers the evidence for *Ulva* having a greater resistance to high light and UVR then *P.palmata*. Reduced RGR (g per day) were observed in *Ulva* (lower) and *P.palmata* (upper and lower) with increasing light intensity. As *Ulva* showed no reduction in growth from upper shore samples it can be denoted that *Ulva* derived from upper shore areas has the ability to acclimate. Increased irradiance and exposure to UV has been seen to induce protective mechanisms and the presence of UVA induces carotenoids and protective mechanisms such as MMAs in red, brown but only a few green algae (Kräbs, *et al.*2002). These are factors which have been seen to govern tolerance to UV and decrease in concentration as depth increases (Hoyer, *et al.* 2001). This protection comes with a twofold trade-off as energy for synthesis is diverted from growth and the accumulation of MMAs results in a lowered photosynthetic ability (Kräbs, *et al.* 2005). However, it has been shown that an increase in carotenoids does not result in a reduction of growth (Jahnke, *et al.* 1999). Further research is needed in order to determine the cause of the decreases in photosynthetic efficiency and growth in both species studied, to determine if MMA can be synthesised and if so, if they can be induced by light, thereby accumulating and increasing protection (Karsten *et al.* 1999). Moreover, the presence of antioxidants, in particular ascorbic acid which is present in higher concentrations in green algae compared to red and brown could be a contributing factor that may be attributed to the resistance of *Ulva* in comparison to *Palmata* (Shiu, and Lee, 2005).

Although, in the short term reductions in growth may occur, the impact on the long term success of the organism will be little affected as beneficial impacts of the protection outweigh the negative. Without protective mechanisms, algae have been seen to experience structural changes to cells, DNA damage and gene alterations which can further decrease the health, reduce reproductive success and eventually cause death (Roldela, *et al.* 2006, 2007).

Changes due to salinity were less obvious than position and light. Further significant decreases in *F*v/*F*m were seen in lower shore *Ulva*, under PARAB and low salinity. This increased the stress of UVB and low salinity proved enough stress to lower the photosynthetic efficiency significantly compared to PAR after the treatment (g per day) having an additive effect.

Generally *Ulva* exhibited lower RGR (g per day) in low than in normal salinities, although only significant in lower shore samples with PARA conditions. *P.palmata* showed increases of RGR (g per day) under PAR and low salinity but decreases under both UVR treatments. This caused significance between the light treatments whereby there was no difference under normal salinity. It can therefore be concluded that the effects are additive in this particular species and collection site of the shore. An unforeseen result was the increased RGR of *Ulva* from the upper shore under low salinity. This could be due to an acclimation of upper shore algae to low/varied salinity conditions and so that under constant salinity a lower fitness is a result.

More obvious changes were seen in pigment concentration under low salinities. Upper shore samples of *Ulva* had higher chlorophyll in normal salinity (PAR/PARA) but in lower shore this switched, meaning both *Ulva* (PAR) and *P.palmata* (PARAB) had increased Chla in low salinities. Furthermore, carotenoid concentration

increases under stress (Jahnke, *et al.* 1999) which was seen in half salinities of samples collected from the lower shore (*Ulva* PAR, *P.palmata* PARAB) which means that lower shore samples have an increased need for protection and are producing increased defence against harmful effects of high light under low salinity. Increasing carotenoid concentration found in the lower salinity samples would result in a greater protection against UVR. Reduced resistance due to other mechanisms of protection not present may also occur, so a need to accumulate carotenoids is paramount. Increased Chla and carotenoids in *P.palmata* did not result in an increased growth or lessen decrease in photosynthetic performance indicating that this could still be resulting in stress to the algae or indicate that an energy trade-off is in force and the possibility of MAAs being present is plausible (Lee, and Shiu, 2009).

In response to the interactive behaviour of UV and salinity, there seems to be a general trend of decreasing fitness under low salinity and increasing light irradiance. In terms of RGR, upper shore *Ulva* increases under low salinity and PAR which indicates the species had adapted well to low salinities under low light. However, when light increases, decreases are seen which are greater than normal salinities so suggest an additive effect. Conversely, the results show the nature of the interaction is highly complex and depends largely on the species, location of collection and thus protective stress mechanisms.

Species which exhibit few or little changes under UV would be considered antagonistic as protective mechanisms such as carotenoids have been seen to increase under low salinity, thus protecting against harmful irradiances. Further research is needed into the action of low salinity and UVR on protective mechanisms and damaging effects on algae in the; structural integrity of cells, ultrastructure and effects of MAAs. This research is required in order to fully understand the mechanisms underlying the interactions between species of algae inhabiting the intertidal zone.

Increasing rainfall over the UK is predicted to increase in the future, with floods and run off from the land into shore areas increasing (Fowler, *et al.* 2005). This coupled with increased light intensity during summer months and the hole in the ozone layer allowing increased UVB through, the focus of this study poses as a very real situation affecting algae worldwide. The implication of these findings to ecosystem and community structure is paramount. Changes in community structure can therefore be expected dependent on the action in which the collective effects have (Bischof, *et al.1998)*. Species with antagonistic interactions would therefore occur higher on the shore and sensitive species (with additive effects) occurring lower. This will ultimately have repercussions on ecosystems and the species which occur within them. Optimistically, from this study low salinity and UVR do not appear to work in synergy, so extended detrimental effects are unlikely.

Conclusion

Low salinity and UVR both have negative effects on the photosynthetic efficiency and growth of both species. The extent is dependent largely on the position of the shore in which the algae inhabit and the species, due to differences in protective mechanisms and tolerance to stress. Under low salinity and PARAB, Chla and carotenoids increase in lower *P.palmata* indicating there is antagonistic actions which cause no change in the RGR from PAR or PARA. Whereas in the upper position salinity it is significantly lower in PARA having an antagonistic effect. *Ulva*

saw significant decreases in photosynthetic efficiency when under salinity stress and UVB in the lower shore samples which were seen to have an additive effect. This could have repercussions on the distribution of species if low salinities and high light become a frequent event with tolerant species of an antagonistic nature dominating upper shore habitats.

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